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<b>(21) International Application Number:</b> PCT/US97/04070 <b>(22) International Filing Date:</b> 14 March 1997 (14.03.97)  <b>(30) Priority Data:</b> 08/616,668 15 March 1996 (15.03.96) US  <b>(71) Applicant:</b> KALAMAZOO HOLDINGS, INC. [US/US]; 3713 West Main Street, P.O. Box 50511, Kalamazoo, MI 39005-0511 (US).  <b>(72) Inventors:</b> GUZINSKI, James, A.; 8814 West H Avenue, Kalamazoo, MI 49009 (US). STEGINK, Larry, J.; 2305 Barney Road, Kalamazoo, MI 49004 (US).  <b>(74) Agent:</b> HUESCHEN, Gordon, W.; 715 The "H" Building, 310 East Michigan Avenue, Kalamazoo, MI 49007 (US).		<b>(81) Designated States:</b> AU, CA, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> SOLID HOP ACID SALT COMPOSITIONS  <b>(57) Abstract</b>  Compositions containing microparticulate potassium salts of the reduced hop isoalpha acids, DHIA and/or HHIA, in a non-supersaturated aqueous solution of potassium salts of the same hop isoalpha acids, which may also contain other hop isoalpha acids such as IA and/or THIA, which is readily convertible to a single-phase solution upon heating to a moderate temperature with agitation for a short time, thereby providing a convenient composition for the introduction of these particular hop acids into a beer without the inconveniences of prior art compositions for effecting such introduction, and a process for production of the said compositions.		

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## SOLID HOP ACID SALT COMPOSITIONS

### BACKGROUND OF THE INVENTION

#### Field of the Invention

5           The art of brewing and more particularly compositions  
containing potassium salts of the hop isoalpha acids DHIA  
and/or HHIA in a non-supersaturated aqueous solution of  
potassium salts of the same hop isoalpha acids which is  
readily convertible to a single-phase solution upon heating  
to a moderate temperature with agitation for a short time,  
10       thereby providing a convenient composition for the  
introduction of these particular hop acids into the brew  
without the inconveniences of prior art compositions for  
effecting such introduction, and a process for production  
of the said compositions.

#### 15       Prior Art

Four types of isoalpha acids are used in the art of  
brewing. These consist of the conventional unreduced  
isoalpha acids (IA) and three hydrogenated forms thereof:  
dihydro, also called rho (DHIA), tetrahydro (THIA), and  
20       hexahydro (HHIA). The hydrogenated forms are made by the  
reduction of the alpha, isoalpha, and beta acids, by  
procedures practiced in commerce.

25       These compounds, having the aforementioned four  
degrees of saturation, called "analogues" in this specifi-  
cation, have distinguishing properties when incorporated  
into a beer, as listed below:

IA: formed during normal wort boil of hops or hop  
extract, or by isomerization outside the kettle, becomes

"light struck" with the formation of odors reminiscent of a skunk.

Hydrogenated forms of IA are: DHIA: forms less foam, less bitter than IA, light stable.

5 THIA: forms more foam than IA, about twice as bitter, light stable.

HHIA: forms more foam than IA, about as bitter, light stable.

10 Each analogue is present as six major forms: cis and trans, each with three different alkyl chains on the position two carbon. A mixture of DHIA and HHIA may therefore contain twelve different forms, each of which can exist as mixtures of optically active isomers. This means that twenty-four different structures are potentially  
15 present in a mixture of DHIA and HHIA.

A description of the prior art follows:

Methods of manufacturing IA, DHIA, THIA, and HHIA are described in Klingel (USP 3,364,265), Westermann (USP 3,965,188), Worden (USP 3,923,897), Stegink (USP  
20 5,296,637), and Hay (USP 5,013,571), as well as by modifications, known to the art, of the processes described in those patents.

25 Solid magnesium chelates of IA, which can be added to the wort when formed in situ in hop pellets, are described in Burkhardt (USP 4,946,691). Humphrey (USP 3,875,316) describes such a chelate of IA, which can be added to a solution of alkaline phosphate to precipitate the magnesium, thereby liberating the IA as an alkali salt in water (Mitchell, British Spec. 1,161,787). Alternatively, Koller  
30 (USP 3,952,061) describes the acidification of the magnesium chelate to form free IA, with subsequent crystallization of the IA from an organic solvent. This crystalline form of IA is not functional in the brewhouse, since its low solubility makes it incapable of forming an  
35 aqueous solution at a useful concentration.

5 Todd (USP 3,486,906) overcame the solubility limitations of aqueous solutions of IA, DHIA, THIA, and HHIA by admixing with propylene glycol or glycerine. This, in particular, overcomes the crystallization of DHIA which often occurs in Westermann's product. Mitchell (USP 3,949,092 and 3,973,052) also showed a totally aqueous alkaline solution of IA.

10 Lupofresh (D.T. Pat. No. 2,919,232) discloses a solid, granular isohumulate which can be added to wort as is, or from which IA can be extracted for addition to beer. Similarly, Klingel, cited above, discloses a freeze dried-sodium isohumulate. Neither of these products have found their way into commercial practice, the single phase solids being very difficult to dissolve.

15 Sweet (USP 3,839,588) discloses a three-phase liquid mixture produced from hops, containing IA.

20 Guzinski (USP 5,200,227) overcame the solubility limits of the hop acids by finding that they had a co-solvent effect within certain specific ranges of their mixtures. These non-precipitating mixtures, however, do not provide the versatility of composition desired for all beers. This invention overcomes the limitations imposed by the ranges described in that patent as well as the unexpected precipitation which we have occasionally  
25 observed in practice. A possible explanation of this variability is the variability of the analogue ratios of the hop acids.

30 The prior art does not show or suggest the readily-soluble microparticles of potassium hop acid salts suspended in an alkaline solution containing dissolved potassium salts of the same hop acids, as disclosed in this application.

Practical Considerations:

35 It is becoming more common to use mixtures of these hop acid analogues in commercial brewing, so as to take

5 advantage of the distinguishing properties of each one. For example, in a non-light stable beer, a mixture of IA and HHIA may be used, IA being cheaper than HHIA, but HHIA contributing superior foam. In a light-stable beer, a  
10 mixture of DHIA and HHIA, or DHIA and THIA, or all three, may be used in various ratios to achieve given foam, cling, and mouth feel properties in a given beer. The desired combination will depend upon the protein, alcohol, and per-cent of non-fermented carbohydrates in the beverage, since the properties of the analogues are affected by the levels of these other constituents.

15 In making a light-stable beer, it is often desirable to add DHIA and/or HHIA to the kettle late in wort boil, so as to provide bacteriostatic protection for the yeast. THIA is not preferred in this application, since it has poor utilization due to losses on trub separated from the wort prior to fermentation. The novel forms of DHIA and HHIA described herein provide superior utilization and assurance of their bacteriostatic effect during fermentation, as compared with the prior-art compositions.

20 When used in the brewhouse, these hop bitter acids are in the form of their potassium salts in aqueous solution, sometimes containing propylene glycol. Maximum commercial concentrations are limited by solubility characteristics. The preferred aqueous preparations commercially available  
25 are as follows: IA-30%; DHIA-35%; THIA-10%. HHIA is not available, since its maximum non-precipitating aqueous solution is only 5%.

30 Upon cooling from ambient to temperatures of 4 to 10°C. often present in the brewhouse or during shipment, IA and THIA preparations may separate into two phases, the lower phase being a more concentrated gum, and the upper a more dilute aqueous solution. In the case of IA and THIA, these gums can be reincorporated by gentle warming and  
35 stirring.

5 Unlike IA and THIA, the 35% commercial preparations of DHIA will often form crystals of DHIA upon standing at ambient temperatures. The crystals of DHIA will not readily redissolve and, when formed, require prolonged heating at elevated temperatures to redissolve. Unless redissolved, they are unavailable to the beer.

10 HHIA, as noted above, does not form solutions in water of practical concentration. Although solutions of 10% or more may be made by heating, they crystallize over time at ambient temperature and require reheating to 80-90°C. for prolonged periods to effect dissolution.

15 Even with prolonged heating, there is no assurance that some of the crystals of DHIA and HHIA will not remain in the bottom of the closed polypropylene container in which they are shipped and from which they are withdrawn. This results not only in unused product, but more significantly makes for non-uniformity in the beer due to variability in the amounts of the hop acids being added to it.

20 One method of achieving non-crystallizing solutions of DHIA and HHIA is described in Guzinski, USP 5,200,227, which utilizes an unexpected co-solvent effect of the different types of isoalpha acids. Although a significant contribution to the art, it shows that mixtures outside the ranges of constituents described therein will form insoluble crystals. Furthermore, we have since noticed that occasionally mixtures within these ranges will crystallize. We suspect that this is due to variations in the ratios of the analogues of the hop acids present, due to variations in the ratios of the acids in the parent hop source, but there is no certain explanation of this deficiency.

30 The present invention provides commercially useful concentrated preparations of DHIA and HHIA which are stable as well as easily used in the brewhouse. It also provides stable concentrated mixtures thereof with IA and THIA.

5 Practical concentrations of hop acids of 50% to 60% are achieved by this invention, the upper concentration being limited by viscosity, which in turn limits pourability. This reduces the amount of plastic requiring disposal or recycling, as well as shipping and warehouse costs. The invention avoids the hazards associated with prolonged heating at near-boiling temperatures and of handling scalding solutions in the brewhouse, as well as the danger of rupture of the polypropylene container during heating.

#### OBJECTS OF THE INVENTION

15 It is an object of the present invention to provide a novel manner of introducing hop isoalpha acids, especially DHIA and/or HHIA, into a brew without the inconvenience of prior art compositions and procedures for effecting such introduction.

20 A particular object is the provision of compositions containing potassium salts of the hop isoalpha acids DHIA and/or HHIA in a non-supersaturated aqueous solution of potassium salts of the same hop isoalpha acids, which are useful for the aforesaid purpose.

25 Another object of the invention is to provide such compositions which are readily convertible to a single-phase solution upon heating to a moderate temperature with agitation for a short time, thereby providing a convenient composition for the introduction of hop isoalpha acids, and especially these particular hop isoalpha acids, into the brew.

30 A still further object of the invention is the provision of a process for the production of such compositions.

Further objects of the invention will become apparent hereinafter and still others will be obvious to one skilled in the art to which this invention pertains.

SUMMARY OF THE INVENTION

What we believe to be our invention, then, inter alia, comprises the following, singly or in combination:

5 A composition comprising a mixture of irregularly-shaped particles of compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) in a liquid at least partially aqueous alkaline solution of potassium salts of the corresponding DHIA or HHIA, which solution is non-supersaturated with respect to the potassium salts of dihydroisoalpha acids (DHIA) or potassium salts of hexahydroisoalpha acids (HHIA) present therein, the size of the particles on their greatest dimension being not more than 250 microns,

15 such a composition which also contains hop essential oil,

such a composition wherein the size of the particles on their greatest dimension is not more than 25 microns, and preferably not more than about 10 microns,

20 such a composition containing particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA),

such a composition containing also dissolved potassium salts of at least one of unreduced isoalpha acids (IA) and tetrahydroisoalpha acids (THIA),

25 such a composition containing particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) and containing also dissolved potassium salts of at least one of unreduced isoalpha acids (IA) and tetrahydroisoalpha acids (THIA),

30 such a composition which becomes a single-phase solution in not more than one hour upon heating to a temperature not greater than about 60°C with agitation,

35 such a composition containing at least one solvent selected from glycerine and propylene glycol, and

such a composition wherein the solvent is present as a minor proportion of the aqueous solution.

Moreover, a process for forming irregularly-shaped microparticles of compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) in which the particles are not more than 250 microns on their greatest dimension in a liquid at least partially aqueous solution which is non-supersaturated with respect to potassium salts of the corresponding DHIA or HHIA, comprising the step of subjecting a starting at least partially aqueous solution which is supersaturated with respect to compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) to agitation, thereby crystallizing out irregularly-shaped microparticles thereof, and maintaining the mixture at ambient temperature until particle formation ceases due to loss of supersaturation by the starting solution,

such a process comprising the step of introducing microparticles of the corresponding compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) into the solution to increase the rate at which supersaturation disappears,

such a process wherein the particles have a particle size not more than 25 microns on their greatest dimension, preferably not more than about 10 microns on their greatest dimension,

such a process wherein the microparticles introduced are not more than 250 microns on their greatest dimension, preferably not more than 25 microns on their greatest dimension,

most especially not more than about 10 microns on their greatest dimension,

5 such a process wherein potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are present in the starting solution and wherein particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are produced;

10 such a process wherein potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are present in the starting solution and wherein particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are produced and wherein microparticles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are introduced into the solution to increase the rate at which supersaturation disappears,

15 such a process wherein the crystal size of the resulting microparticles is reduced by mechanical means to eliminate crystals more than about 25 microns in size on their greatest dimension,

20 such a process wherein the crystal size of the resulting microparticles is reduced by mechanical means to eliminate crystals more than about 25 microns in size on their greatest dimension to less than about 20% of the total of all microparticles present, and

25 such a process wherein the aqueous solution contains at least one solvent selected from glycerine and propylene glycol.

#### GENERAL DESCRIPTION OF THE PRESENT INVENTION

##### THE PRESENT INVENTION:

30 There are three elements critical to this invention: (1) the liquid phase in contact with DHIA and/or HHIA be non-supersaturated with these constituents, (2) the solid particles of DHIA and/or HHIA have a size not more than 250 microns on their greatest dimension, preferably not more than 25 microns, and even more preferably not more than  
35 about 10 microns on their largest dimension, and are

irregular in shape, and (3) the liquid phase must be alkaline and the hop acids in both the liquid and solid phases must be present as their potassium salts. Such a composition is heretofore unknown to the art.

5 We have found that the single-phase solutions from which crystals of DHIA and HHIA form are supersaturated, and that at unpredictable times crystallization may be spontaneously initiated. It will continue for periods as long as a year or more, usually in the bottom of the  
10 container, forming hard insoluble crystals of up to a centimeter or more in size. The composition can then only be liquified by prolonged heating at temperatures in the range of 90°C.

The process for overcoming supersaturation preferably  
15 involves adding seed particles to a supersaturated solution, thereby initiating formation of microparticles in the supersaturated solution. The seed may be prepared by the reduction of crystal size from the large crystals, which develop in the DHIA and HHIA solutions now commercially available, to microcrystals of not greater than 250  
20 microns, and preferably not greater than 25 microns in size. Or the seed may be a previously-prepared microparticle suspension. Although formation of microparticles can be effected by agitation of a supersaturated solution without prior seeding, this is a much less preferred manner  
25 of operation because particle size is not easily controlled in such case, nor does the crystallization occur within a predictable period of time. It is critical that agitation of the supersaturated solution continue for a sufficient  
30 period to assure that supersaturation of the solution does no longer exist, whether the solution be seeded or unseeded.

This simple but novel process produces a product  
35 containing microparticles of smaller average dimension than the seed, as demonstrated in Example 7. This is contrary to

the accepted teachings of the art of crystallization, for loss of supersaturation should be accompanied by growth of the seed. Rather than growing on the added microparticles, the new particles appear to be formed de-novo. Furthermore, the particles, by the time supersaturation has ceased to exist, are of undefined shapes and appear amorphous, rather than crystalline. It is surmised that they are co-solidified mixtures of the various analogues and optical isomers, rather than pure crystals.

The resulting suspension of microparticles in a non-supersaturated aqueous medium is then readily redissolved by gentle warming and agitation, producing a solution of DHIA and HHIA which is easy to add to the beer.

Conjecture as to the explanation of the efficacy and novelty of the product of this invention follows. The effects and importance of supersaturated solutions of hop acids has not heretofore been reported or recognized in the art. Depending upon unknown factors, the crystallization usually begins after a month to several months, and continues for a year or more. The crystals have "clean" planes when examined under the microscope, and are up to 1 cm in size. When pulverized to form seed, they retain clearly defined shapes. However, when such seed is added to a supersaturated solution, it causes the analogues of the hop acids to congeal into solids of mixed composition and undefined shapes and, for some unknown reason and contrary to expectation, these new particles are smaller in average size than the seed, as well as irregular in shape. Because they are not crystals of pure compound, but rather mixed aggregates, they redissolve more readily than pure crystals, even of the same size.

The novel, two-phase, liquid plus solid, non-supersaturated solution of DHIA and HHIA containing microparticles of the hop acids will remain stable for an indefinite period of time. All of the hop-derived acids become

available to the beer with gentle manipulation in the brewhouse. This liquid plus solid, two-phase suspension of hop acids in a non-supersaturated medium which also contains dissolved hop acids is new to the art. Hop essential oil may be added to the mixture to contribute hoppy aroma to the flavored beer.

It is known that the analogues present vary from hop variety to hop variety, as well as the method of manufacture of DHIA and HHIA, and the behavior of hop acids prepared from different hops or by different procedures will vary. This invention, however, is equally applicable to all forms of the acids.

The present invention has its greatest present utility in overcoming the deficiencies of aqueous solutions, but it is equally applicable to such solutions which also contain propylene glycol and/or glycerine.

#### DETAILED DESCRIPTION OF THE INVENTION

The following Examples are given by way of illustration only, and are not to be construed as limiting.

#### Examples

Dihydro-isoalpha acids (DHIA), tetrahydro-isoalpha acids (THIA), hexahydro-isoalpha acids (HHIA), and isoalpha acids (IA) used in these examples were obtained as commercial products. A small amount of the reduced hop acids (DHIA, THIA and HHIA) was purified by liquid chromatography on silica gel to produce the seed. Alternatively, crystals were removed from stored commercial products and ground to less than about 25 microns, and most to less than 10 microns, for use as seed.

#### Example 1.

Illustration of supersaturated solutions of the prior art and non-supersaturated solid-liquid phase mixtures containing microparticles which have greatly enhanced solubility, and process for making them.

5 A single phase pH 9.5 aqueous solution containing 20% HHIA and 10% THIA was prepared. It required heating to 80°C. and stirring for several hours to dissolve the crystals which were present. This mixture was separated into three portions.

10 A. One portion was stored at room temperature for two months. During this time a thick layer of crystals of the hop acids formed in the bottom of the container. To redissolve this layer of crystals required heating to about 85°C. with vigorous agitation for six hours. This is the mixture of the prior art having poor solubility properties and requiring heating and agitation to an extent inherently unsafe and impractical in a brewery.

15 B. The second portion was seeded with crystals of the potassium salts of HHIA and THIA acids having a maximum dimension of less than 25 microns, made as above, and stirred for 24 hours at ambient. A large quantity of micro-  
20 particles formed. This portion was stored at room temperature for two months. Examination under a microscope revealed that the particles were less than about 250 microns and most were less than 25 microns in size. The microparticles occurred in a mixture of shapes unlike the shapes of the parent seed crystals: platelets, irregular  
25 needles, irregular rhomboids, and amorphous solids were observed. This mixture completely dissolved when heated to 58°C for five minutes with gentle agitation. The solid-liquid mixture containing the microparticles dissolved completely within five minutes when added to 50°C. water at a pH of 10, forming a clear solution of 1% hop acids.  
30 Since pre-dilution of hop acid composition with alkaline water is a common brewhouse technique, this example shows the inventive product to be compatible with normal brewhouse practice.

Further storage of the mixture at 5°C. for two months did not cause agglomeration, size increase of the microparticles, or other physical change.

5 C. The third portion of the original supersaturated mixture was stored at room temperature for two months. During this time a thick layer of crystals of the hop acids formed, as in Example (1A) above. This portion was then milled to reduce the particle size to less than 25 microns. The mixture was then stirred at room temperature for 24  
10 hours. During the stirring time, there was a large increase in the number of visible particles. Microscopic examination of the particles showed a mixture of different particle types as in Example 1B. The mixture completely dissolved when heated to 60°C. for five minutes with gentle stirring.  
15 This solid-liquid mixture containing microparticles is, like the product of Example 1B, very easy to use in a brewery.

The large increase in the number of particles after stirring also shows the further changes which occur with  
20 this procedure. Measurement of the hop acid concentration in the liquid phases was made by UV analysis according to procedures well known to the art. This showed that the original solution was at a concentration of 30.0%, as expected. It decreased to 28.4% after two months with the  
25 formation of large crystals. After being milled and restirred, the liquid portion of the mixture, separated by centrifugation, decreased further to 24.2%. This mixture was stable as to further crystallization.

It is noticed that about 5% of the hop acids crystal-  
30 lized from the original 30% solution, to give the 28.4% solution. An additional 14% of the acids precipitated due to the seeding and agitation. Almost three times the amount of hop acids was removed by the procedure from the still supersaturated 28.4% solution as was removed during the  
35 standing of the original solution for two months. It is

unexpected that a solution, which had been allowed to crystallize for two months and would be assumed to have reached equilibrium with respect to crystallization, would produce such a large amount of micro-particulate solids in an additional 24 hour period, and no longer be supersaturated.

Example 2. A soluble mixture of reduced hop bitter acids of 45% concentration.

A. A mixture of 20% DHIA, 20% HHIA and 5% THIA was dissolved at 90°C. in alkaline water with the pH adjusted to 10.5 with potassium hydroxide. The solution was cooled to ambient temperature and seeded with the previously-prepared <25 micron crystalline potassium salts of DHIA, HHIA, and THIA and stirred overnight. A dispersion of fine particles formed which stayed in suspension; the appearance of the mixture was similar to a liquid crystal. Examination by microscope showed the microparticles to be a mixture of plates, irregular rhomboid structures, fine irregular needles, and amorphous solids having their largest dimensions not greater than 250 microns, and most less than 25 microns. After two months in storage at room temperature, the mixture had not shown any settling of the microparticles or development of large crystals.

B. The seeded mixture from A, with a total hop acid concentration of 45%, was readily soluble when heated to 51°C. for a few minutes. This heated solution, when then added to ambient temperature distilled water raised to pH 10 with KOH, gave crystal-clear solutions at 1% and 5% concentration of hop bitter acids.

C. The mixture from A containing microparticles promptly dissolved in 45 C. pH 10 water at 1%, with no visible particles, to give a clear solution.

This example also demonstrates the practicality of liquid-solid mixtures of hop acids at high concentrations.

Such concentrations, previously unavailable, reduce packaging, shipping, and storage costs.

Example 3. A mixture of reduced hop bitter acids with propylene glycol, a common edible adjuvant.

5           The mixture from Example 2 was diluted by the addition of 5% by weight of propylene glycol. There was no change in appearance of the mixture except for a decrease in viscosity. This mixture redissolved upon warming similarly to the mixture of Example 2, from which it was derived. A  
10       cosolvent such as propylene glycol or glycerine can be used as part of the composition if reduction of viscosity or freezing point is desirable. A minor proportion of such solvent, e.g., less than about 40% by volume of the aqueous solution, is preferred.

15       Example 4. A mixture containing HHIA with high solubility.

          Large crystals of the potassium salts of hexahydro-isoalpha acids were collected from an aqueous alkaline product containing HHIA. A solution was made by dissolving these crystals in water with addition of potassium  
20       hydroxide, to a final pH of 10.6 and a hop acid concentration of 20%, which required heating to 90 C. with vigorous agitation for several hours. The solution was cooled with vigorous stirring. During cooling, a fine precipitate formed. The cooled mixture passed through a 60 mesh (250  
25       micron) screen. The majority of the particles were in the range of 10 to 25 microns in size, irregular, and roughly ovoid in shape although some smaller flat plates were visible. Some were starburst in appearance. A significant percentage of the particles were less than 10 microns on  
30       their largest dimension. There were no distinct planes or edges visible on the particles, such as would be formed from pure crystals. HHIA is a mixture of six major forms of the acids, differing in alkyl side chains and being present as cis and trans isomers. It contains optical isomers of  
35       all these forms. Rapid crystallization or precipitation

5 may not allow fractional crystallization and separation of  
analogous and isomers, thereby inhibiting the formation of  
crystals with uniform surfaces which are resistant to  
redissolution. Surprisingly, the microparticles did not  
have the same sharp edges as the original, ground, seed  
crystals.

10 A 1% HHIA solution was made by diluting the mixture in  
19 parts of distilled water to which sufficient dilute  
potassium hydroxide had been added to increase the pH to  
11.0. Although fine particles were visible at ambient  
temperature, all the particles promptly dissolved when the  
1% dilution was heated to about 50 to 55°C. In contrast, the  
parent large crystals, when added to alkaline water so as  
to effect a 1% concentration, did not dissolve at 75°C.  
15 when stirred for four hours.

This Example demonstrates that a mixture of different  
types of hop acids is not necessary for implementation of  
this invention.

20 This mixture did not agglomerate and the fine  
particles, which settled upon storage, were readily  
resuspended by inverting the container. Storage for a  
period of two months did not induce agglomeration or  
hardening of the microparticulate HHIA, nor did formation  
of insoluble crystals occur.

25 The 20% concentration of HHIA in this example is in no  
way a limiting concentration. Higher concentrations, even  
greater than 50%, can be obtained by using a greater  
percentage of the potassium salts of HHIA in the solution  
made at the high initial temperature before cooling and  
30 stirring to form the microparticles.

While this example shows that microparticles of HHIA  
can be made without seeding, in practice seeding is  
desirable to assure that supersaturation does not occur.  
Furthermore, it has been observed that the rate of micro-  
35 particle formation, in the absence of seeding, is unpre-

dictable. Therefore seeding is preferred in practice.

Example 5.

5 An aqueous solution was made containing 50% IA and 33% glycerine at a pH of 9.2, using aqueous KOH. One part by weight of this solution was added to one part of the microparticle-containing mixture of Example 1B. This gave a mixture containing 25% IA, 10% HHIA, 5% THIA and 16.5% glycerine, the glycerine effectively reducing the viscosity. This microparticle-containing mixture promptly  
10 dissolved at 59°C. and, when so dissolved, readily and completely dissolved in pH 10 water to form a convenient 1% solution of hop acids. This demonstrates a practical method to add the foam-enhancing properties of THIA and HHIA to produce a product containing non-reduced hop acids IA. Such  
15 a composition enables a brewer to use a highly concentrated product for both bitterness and improved foam.

This Example also demonstrates the use of the novel products of microparticulate hop acids when added to and used in conjunction with a hop acid that does not readily  
20 form crystals or solid particles. Commercial 30% IA is not known to form crystals, although separation of the hop acid as a thick gum from refrigerated product is common.

Example 6. A mixture of microparticulate and liquid hop bitter acids containing hop oil.

25 An aqueous mixture was made containing 20% HHIA, 10% THIA and 0.6% hop oil at pH 10.6. The mixture was heated to 85°C. to effect dissolution of the solid hop acids. After cooling to ambient temperature, during which period crystallization did not occur, a small amount of the microparticulate-containing mixture from Example 1B was  
30 added and this mixture stirred for 48 hours. A slurry of fine microparticles formed. The hop oil was not visible as an oil slick on the surface of the mixture, as would be expected since hop oil is insoluble in water. The solubility properties of this mixture were the same as in Example  
35

1B.

Although it is not known if the hop oil is entrained in the microparticles, adsorbed on the surface of the particles, hydrophobically bonded to dissolved hop acids, or otherwise held in the mixture, the hop oil does not separate out and can, therefore, be incorporated into beer simultaneously with the hop acids. This provides a convenient, novel, and useful form of total hop flavor, both bitterness and aroma, in brewery applications.

Example 7.

An originally single-phase mixture consisting of 20% HHIA and 10% THIA, prepared by mixing the ingredients in alkaline water at pH 10.5 and heating to about 90°C. until a single-phase liquid formed, was cooled to ambient temperature. It remained in a single phase during the cooling and subsequent packaging into containers for warehouse storage. When placed in a warehouse at 20°C., it remained a single-phase liquid. After two and one-half months at 20°C, a heavy precipitate had formed on the bottom of the container. The assay of the liquid phase above the solids had decreased from 29.8% originally to 28.8% during storage, due to the precipitation. The product required heating to 90°C. for several hours to redissolve.

This product was milled, using procedure known to the art, to reduce particle size to essentially less than 250 microns, with an average particle size of 23 microns. It was agitated for a day, a sample withdrawn, and agitation continued for another two days. Viscosity increased as more microparticles formed. The liquid-solid mixture contained 29.8% hop acids, the same as the original assay. The supernatant, separated by centrifugation, had decreased to 26.4% after one day, and to 24.7% after two additional days. This shows that even after two and one half months, the original product was supersaturated, and would continue to form insoluble crystals.

This product containing microparticles does not form insoluble crystals on standing, since it is no longer supersaturated.

5 A high performance liquid chromatogram (HPLC) of the solids showed a distribution of analogues and isomers of HHIA different from the original supersaturated mixture, with only a trace of THIA. The solids had irregular shapes and did not show clean linear crystal faces. The analysis of particle size was performed on a Lasentec M100 FBRM  
10 (Focused Beam Reflectance Measurement) System. Focal depth was set at 0.0 (focused at the windows) for all measurements. The stirrer was set at 500, and measurements were made at ambient temperature. Distributions were reported as unweighted mean chord lengths. Particle sizes are  
15 considered to be two-thirds of the measured chord length, an approximation considered valid for systems of particles which are roughly spherical in shape. In this manner, it was determined that particles averaged 23 microns in size following milling and, after agitating a day, the average  
20 size became 12 microns as new microparticles formed. The size distribution was unchanged after three days. It should be noted that this reduced average included all of the original microparticles averaging 23 microns in size.

Ordinarily, it would be expected that particle size  
25 would grow due to deposition of hop acids on the seed during the day of agitation, but it is clear that this did not occur and that, after the original crystal size reduction, followed by agitation to form the irregularly-shaped microparticles, the average size of the particles  
30 decreased. This is a novel and unexpected result, and therefore makes it possible either to mill existing crystals, or to introduce seed particles, and achieve a reduction in average particle size in the supersaturated solution as it loses its supersaturation.

5 The mixture of microparticles itself did not redissolve until a temperature of 70°C. was reached, whereupon it dissolved promptly (as opposed to 90°C. and several hours of agitation for the stored product from which it was made). However, when the mixture was diluted 25% with water, and that suspension was warmed, dissolution occurred promptly when a temperature of 45°C. was reached. This shows that the solubility limits of HHIA (and DHIA as well) in an alkaline non-supersaturated solution of their potassium salts controls the redissolution temperature and that, as more water is added to the mixture, the redissolution temperature decreases. With sufficient water, this mixture of microparticles redissolves promptly at 30 to 40°C.

10 By way of further illustration, it should be noted that the 30% mixture, redissolved at 70°C., becomes supersaturated upon cooling. Without agitation and seeding, it remains supersaturated for an indefinite period of time, and then only slowly regrows large crystals in Example 7, as occurred in the containers in the warehouse in Example 7.

20 Example 8. A 51.3% mixture of DHIA microparticles in a non-supersaturated solution of DHIA.

A 51.3% non-supersaturated solution of DHIA was prepared by heating it to 85°C. in water, with the pH adjusted to 9.2 with KOH. This solution became supersaturated upon cooling, but did not immediately form solids. Upon stirring at ambient for four days, it became opalescent with the appearance of liquid crystals in a mother liquor. Examination under a microscope showed that it contained a mixture of particle shapes. Some particles had the appearance of needles, a few of about 25 microns in length, but most less than 10 microns in length. The majority of the particles were amorphous, with an average largest dimension of less than 10 microns. The mixture was

stable over a six-week period, the microparticles being so small that little settling was observed.

5       The 51.3% DHIA dissolved in pH 10.5 water when diluted to a 1% concentration, at ambient temperature, within a few minutes. Alternatively, the mixture formed a non-supersaturated solution upon warming to 47°C.. This warm solution formed a clear solution when added to pH 10.5 water at a 1% DHIA concentration.

10       The prior art 35% supersaturated solution containing crystalline DHIA does not totally dissolve or form clear solutions when added to pH 10.5 water under the above conditions. While dilution of the 51.3% solution to 35% would provide a product of similar concentration and superior utility to the present art product of limited  
15       solubility, the ability to provide a more concentrated product with improved solubility demonstrates the advantage of this invention.

Example 9. Direct Dosing of microparticulate mixtures into beer.

5       The 30% THIA-HHIA microparticle mixture of Example 7 was diluted to 1% in pH 10 water at ambient temperature, forming a hazy solution. This was immediately dosed into a beer in a clear glass bottle at a rate to add 15 ppm of hop acids to the beer. The bottle was recapped and inverted to mix the injecta, which made the beer hazy. It was then pasteurized. Upon removal from pasteurization it had become clear, and remained clear upon cooling to ambient.

10       Hop acid crystals were removed from the bottom of the warehouse-stored containers of Example 7. 10 mg of these crystals were added to a bottle of the beer used above, the bottle recapped, and pasteurized. The crystals had not gone into solution, and could be seen suspended in the beer.

15       This example shows that the microcrystalline mixture of this invention may be directly injected into beer, preferably prediluted, whereas the mixture of the prior art, containing large crystals as represented by the warehouse-stored product of Example 7, cannot be so injected.

\* \* \* \* \*

20       It is to be understood that the present invention is not to be limited to the exact details of operation, or to the exact compounds, compositions, methods, procedures, or embodiments shown and described, as various modifications and equivalents will be apparent to one skilled in the art, wherefore the present invention is to be limited only by the full scope which can be legally accorded to the appended claims.

WE CLAIM:

- 1 -

5 A composition comprising a mixture of irregularly-shaped particles of compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of  
10 hexahydroisoalpha acids (HHIA) in a liquid at least partially aqueous alkaline solution of potassium salts of the corresponding DHIA or HHIA, which solution is non-supersaturated with respect to the potassium salts of  
15 dihydroisoalpha acids (DHIA) or potassium salts of hexahydroisoalpha acids (HHIA) present therein, the size of the particles on their greatest dimension being not more than 250 microns.

- 2 -

15 A composition of claim 1 wherein the size of the particles on their greatest dimension is not more than 25 microns.

- 3 -

20 A composition of claim 1 wherein the size of the particles on their greatest dimension is not more than about 10 microns.

- 4 -

25 A composition of claim 1 containing particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA).

- 24 -

- 5 -

A composition of claim 1 containing also dissolved potassium salts of at least one of unreduced isoalpha acids (IA) and tetrahydroisoalpha acids (THIA).

5

- 6 -

A composition of claim 1 containing particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) and containing also dissolved potassium salts of at least one of unreduced iso-

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- 7 -

A composition of claim 1 which becomes a single-phase solution in not more than one hour upon heating to a temperature not greater than about 60°C with agitation.

15

- 8 -

A composition of claim 1 containing at least one solvent selected from glycerine and propylene glycol.

- 9 -

A composition of claim 8 wherein the solvent is present as a minor proportion of the aqueous solution.

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- 10 -

A process for forming irregularly-shaped microparticles of compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) in which the particles are not more than 250 microns on their greatest dimension in a liquid at least partially aqueous solution which is non-supersatu-

25

- 25 -

rated with respect to potassium salts of the corresponding DHIA or HHIA, comprising the step of subjecting a starting at least partially aqueous solution which is supersaturated with respect to compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) to agitation, thereby crystallizing out irregularly-shaped microparticles thereof, and maintaining the mixture at ambient temperature until particle formation ceases due to loss of supersaturation by the starting solution.

- 11 -

A process of claim 10 comprising the step of introducing microparticles of the corresponding compounds selected from potassium salts of dihydroisoalpha acids (DHIA) and potassium salts of hexahydroisoalpha acids (HHIA) into the solution to increase the rate at which supersaturation disappears.

- 12 -

A process of claim 10 wherein the particles have a particle size not more than 25 microns on their greatest dimension.

- 13 -

A process of claim 10 wherein the particles have a particle size not more than about 10 microns on their greatest dimension.

- 26 -

- 14 -

A process of claim 11 wherein the microparticles introduced are not more than 250 microns on their greatest dimension.

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- 15 -

A process of claim 11 wherein the microparticles introduced are not more than 25 microns on their greatest dimension.

- 16 -

10

A process of claim 11 wherein the microparticles introduced are not more than about 10 microns on their greatest dimension.

- 17 -

15

A process of claim 10 wherein potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are present in the starting solution and wherein particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are produced.

- 18 -

20

A process of claim 11 wherein potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are present in the starting solution and wherein particles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA) are produced and wherein microparticles of potassium salts of both dihydroisoalpha acids (DHIA) and hexahydroisoalpha acids (HHIA)

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- 27 -

are introduced into the solution to increase the rate at which supersaturation disappears.

- 19 -

5 A process of claim 10 wherein the crystal size of the resulting microparticles is reduced by mechanical means to eliminate crystals more than about 25 microns in size on their greatest dimension.

- 20 -

10 A process of claim 10 wherein the crystal size of the resulting microparticles is reduced by mechanical means to eliminate crystals more than about 25 microns in size on their greatest dimension to less than about 20% of the total of all microparticles present.

- 21 -

15 A process of claim 17 wherein the crystal size of the resulting microparticles is reduced by mechanical means to eliminate crystals more than about 25 microns in size on their greatest dimension.

- 22 -

20 A process of claim 17 wherein the crystal size of the resulting microparticles is reduced by mechanical means to eliminate crystals more than about 25 microns in size on their greatest dimension to less than about 20% of the total of all microparticles present.

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- 28 -

- 23 -

A process of claim 10 wherein the aqueous solution contains at least one solvent selected from glycerine and propylene glycol.

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- 24 -

A process of claim 11 wherein the aqueous solution contains at least one solvent selected from glycerine and propylene glycol.

- 25 -

10

A composition of claim 1 which also contains hop essential oil.

- 29 -

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/04070**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12C 3/00

US CL : 426/592, 600; 568/341

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/592, 600; 568/341

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,200,227 (GUZINSKI et al) 06 April 1993, examples 1, 6 and 7.	1-25
Y	US, 3,765,903 (CLARKE et al) 16 October 1973, col. 1, lines 23-30, 54-60, col. 2, lines 14-19, col. 3, lines 56-64 and col. 4, lines 19-39.	1-25
Y	Concise Encyclopedia Chemistry, Walter de Gruyter, New York, 1994, pp. 286-87.	10-24

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

Special categories of cited documents:	
*A* document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention
*E* earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* documents referring to an oral disclosure, use, exhibition or other means	*Z* document member of the same patent family
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 16 JUNE 1997	Date of mailing of the international search report <b>09 JUL 1997</b>
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